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=> s chimeric beta lactamase and mimetope and substrate

L1             0 CHIMERIC BETA LACTAMASE AND MIMETOPE AND SUBSTRATE

=> s chimeric beta lactamase and mimetope

L2             0 CHIMERIC BETA LACTAMASE AND MIMETOPE

=> s chimeric beta lactamase and and target molecule

MISSING TERM 'AND AND'
The search profile that was entered contains a logical
operator followed immediately by another operator.

=> s chimeric beta lactamase and target molecule

L3             0 CHIMERIC BETA LACTAMASE AND TARGET MOLECULE

=> s variant beta lactamase and target molecule

L4             0 VARIANT BETA LACTAMASE AND TARGET MOLECULE

=> s (variant or mutata? or mutant) beta lactamase and target molecule

MISSING OPERATOR MUTANT) BETA
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s (variant or mutata? or mutant) and beta lactamase and target molecule

L5             0 (VARIANT OR MUTATA? OR MUTANT) AND BETA LACTAMASE AND TARGET
                 MOLECULE

=> s (variant or mutata? or mutant) and beta lactamase

L6          1043 (VARIANT OR MUTATA? OR MUTANT) AND BETA LACTAMASE

=> dup rem l6

PROCESSING IS APPROXIMATELY  93% COMPLETE FOR L6
PROCESSING COMPLETED FOR L6
L7           653 DUP REM L6 (390 DUPLICATES REMOVED)

=> s l7 and fusion

L8            40 L7 AND FUSION
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=> dup rem l8

PROCESSING COMPLETED FOR L8
L9 40 DUP REM L8 (0 DUPLICATES REMOVED)

=> d l9 1-15 ibib ab

L9 ANSWER 1 OF 40 MEDLINE
ACCESSION NUMBER: 2000100755 MEDLINE
DOCUMENT NUMBER: 20100755
TITLE: Expression of the multidrug resistance transporter NorA from Staphylococcus aureus is modified by a two-component regulatory system.
AUTHOR: Fournier B; Aras R; Hooper D C
CORPORATE SOURCE: Infectious Disease Division and Medical Services, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114-2696, USA.
CONTRACT NUMBER: AI23988 (NIAID)
SOURCE: JOURNAL OF BACTERIOLOGY, (2000 Feb) 182 (3) 664-71. Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200004
ENTRY WEEK: 20000402
AB To dissect genetically the regulation of NorA, a multidrug transporter of Staphylococcus aureus, we analyzed the differential expression of the norA promoter using a transcriptional **fusion** with a **beta-lactamase** reporter gene. Expression studies with an arlS **mutant** revealed that the norA promoter is ArlS dependent. The arlR-arlS locus was shown to code for a two-component regulatory system. The protein ArlR has strong similarity to response regulators, and ArlS has strong similarity to protein histidine kinases. We have also analyzed the 350-bp region upstream of the Shine-Dalgarno sequence of norA by gel mobility shift experiments. It was shown that only the 115-bp region upstream of the promoter was necessary for multiple binding of an 18-kDa protein. From transcriptional fusions, we have localized four different putative boxes of 6 bp, which appear to play a role in the binding of the 18-kDa protein and in the up-regulation of norA expression in the presence of the arlS mutation. Furthermore, the gel mobility shift of the 18-kDa protein was modified in the presence of the arlS mutation, and the arlS mutation altered the growth-phase regulation of NorA. These results indicate that expression of norA is modified by a two-component regulatory system.

L9 ANSWER 2 OF 40 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:718875 CAPLUS
DOCUMENT NUMBER: 131:348774
TITLE: Tandem fluorescent protein constructs and their preparation for enzyme assays
INVENTOR(S): Tsien, Roger Y.; Heim, Roger; Cubitt, Andrew
PATENT ASSIGNEE(S): The Regents of the University of California, USA; Aurora Biosciences Corporation
SOURCE: U.S., 33 pp., Cont.-in-part of U.S. Ser. No. 594,575. CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5981200	A	19991109	US 1997-792553	19970131
PRIORITY APPLN. INFO.:			US 1996-594575	19960131

AB This invention provides tandem fluorescent protein construct including a donor fluorescent protein moiety, an acceptor fluorescent protein moiety and a linker moiety that couples the donor and acceptor moieties. The donor and acceptor moieties exhibit fluorescence resonance energy transfer which is eliminated upon cleavage. The constructs are useful in enzymic assays. **Mutant** green fluorescent proteins (GFPs) were created by mutagenesis of the Aequorea victoria GFP. Polyhistidine tagged tandem green and blue fluorescent proteins were recombinantly constructed having an inserted peptide sequence including cleavage recognition sites for many proteases. Cleavage expts. were done with trypsin, enterokinase and calpain.

L9 ANSWER 3 OF 40 MEDLINE
ACCESSION NUMBER: 1999211973 MEDLINE
DOCUMENT NUMBER: 99211973
TITLE: Secretory production of recombinant protein by a high cell density culture of a protease negative **mutant** Escherichia coli strain.
AUTHOR: Park S J; Georgiou G; Lee S Y
CORPORATE SOURCE: Department of Chemical Engineering and BioProcess Engineering Research Center, Korea Advanced Institute of Science and Technology, 373-1 Kusong-dong, Yusong-gu, Taejon 305-701, Korea.
SOURCE: BIOTECHNOLOGY PROGRESS, (1999 Mar-Apr) 15 (2) 164-7. Journal code: ALG. ISSN: 8756-7938.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907

AB Several protease negative **mutant** strains including HM114, HM126, and HM130 as well as their parent strain KS272 were compared for their growth and secretory production of a model **fusion** protein, protein A-**beta-lactamase**. HM114, a strain deficient in two cell envelope proteases, grew slightly faster and produced more **fusion** protein than the other strains deficient in more proteases. HM114 was grown to a cell dry weight of 47.86 g/L in 29 h using pH-stat, fed-batch cultivation. The **beta-lactamase** activity was 11.25 x 10(4) U/L, which was 30% higher than that obtained with its parent strain KS272. Up to 96% of protein A-**beta-lactamase** **fusion** protein could be recovered by a simple cold osmotic shock method. The specific **beta-lactamase** activity obtained with HM114 after fractionation was 4.5 times higher than that obtained with KS272.

L9 ANSWER 4 OF 40 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:745098 CAPLUS
DOCUMENT NUMBER: 130:13213
TITLE: Recombinant single-chain antibody-.**beta**.-**lactamase fusion** protein targets melanoma cells
INVENTOR(S): Siemers, Nathan O.; Yarnold, Susan; Senter, Peter D.

INVENTOR(S): Siemers, Nathan O.; Yarnold, Susan; Senter, Peter D.
PATENT ASSIGNEE(S): Bristol-Myers Squibb Co., USA
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9850432	A1	19981112	WO 1998-US8840	19980430
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9872748	A1	19981127	AU 1998-72748	19980430
PRIORITY APPLN. INFO.:			US 1997-45888	19970507
			US 1998-70637	19980430
			WO 1998-US8840	19980430

AB The authors disclose a recombinant **fusion** polypeptide comprising antibody VH and VL sequences operatively linked in a single-chain scFv format to **.beta.-lactamase**. The scFv-**.beta.-lactamase fusion** protein recognized the tumor-assocd. antigen melanotransferrin and exhibited biol. activity against melanoma cells in conjunction with the cephalosporin mustard prodrug CCM.

L9 ANSWER 5 OF 40 MEDLINE
ACCESSION NUMBER: 1998233734 MEDLINE
DOCUMENT NUMBER: 98233734
TITLE: Ferric citrate transport of Escherichia coli: functional regions of the FecR transmembrane regulatory protein.
AUTHOR: Welz D; Braun V
CORPORATE SOURCE: Mikrobiologie/Membranphysiologie, Universitat Tubingen, Germany.
SOURCE: JOURNAL OF BACTERIOLOGY, (1998 May) 180 (9) 2387-94.
Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199808
ENTRY WEEK: 19980801

AB Transcription of the ferric citrate transport genes of Escherichia coli is induced by ferric citrate bound to the outer membrane receptor FecA. Additional ferric citrate-specific regulatory proteins are FecR in the cytoplasmic membrane and the FecI sigma factor in the cytoplasm. To further understand the assumed FecR-mediated signal transduction across the cytoplasmic membrane, the transmembrane topology of FecR (317 amino acids) was determined with hybrid proteins containing portions of FecR and mature BlaM **beta-lactamase**. BlaM fused to FecR regions extending from residues 107 to 149 and residues 230 to 259 conferred high ampicillin resistance to cells, while BlaM fused to sites between residues 159 and 210 and between residues 265 and 301 conferred low resistance. Cells that synthesized FecR'-BlaM with **fusion** joints between

Cells that synthesized FecR'-BlaM with **fusion** joints between residues 8 and 81 of FecR were fully sensitive to ampicillin. The ampicillin resistance of the low-resistance FecR'-BlaM hybrids was increased 2- to 10-fold by cosynthesis of plasmid-encoded GroEL GroES and SecB chaperones and in degP and ompT protease mutants, which suggested that the decreased ampicillin resistance level of these hybrids was caused by the formation of inclusion bodies and proteolytic degradation. Replacement of glycine by aspartate residues in the only hydrophobic FecR sequence (residues 85 to 100) abolished the **beta-lactamase** activity of high-resistance FecR'-BlaM proteins, indicating that there are no other transmembrane regions in FecR that translocate BlaM into the periplasm independent of the hydrophobic sequence. All FecR'-BlaM proteins with at least 61 FecR residues complemented a fecR **mutant** such that it could grow on ferric citrate as the sole iron source and induced fecA-lacZ transcription independent of ferric citrate. The low resistance mediated by two FecR'-BlaM proteins in a fecA deletion **mutant** was increased 20-fold by transformation with a fecA-encoding plasmid. We propose that FecR spans the cytoplasmic membrane once, interacts in the periplasm with its C-terminal region with FecA occupied by ferric citrate, and transmits the information through the cytoplasmic membrane into the cytoplasm, where it converts FecI into an active sigma factor.

L9 ANSWER 6 OF 40 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:4233 CAPLUS
DOCUMENT NUMBER: 130:163702
TITLE: Efficient recovery of secretory recombinant proteins from protease negative **mutant** Escherichia coli strains
AUTHOR(S): Park, Si Jae; Lee, Sang Yup
CORPORATE SOURCE: Department of Chemical Engineering and BioProcess Engineering Research Center, Korea Advanced Institute of Science and Technology, Taejon, 305-701, S. Korea
SOURCE: Biotechnol. Tech. (1998), 12(11), 815-818
CODEN: BTECE6; ISSN: 0951-208X
PUBLISHER: Chapman & Hall
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Osmotic shock and lysozyme/EDTA methods were used to recover secreted recombinant proteins from protease neg. **mutant** strains of E. coli. Up to 80% of protein A-.**beta**.-**lactamase fusion** protein was recovered from protease neg. mutants by simple osmotic shock. Fractionation by lysozyme/EDTA treatment, increased the recovery of protein A-.**beta**.-**lactamase fusion** protein from the **mutant** strain up to 93%. Mild fractionation condition allowed efficient recovery of secreted protein from protease neg. **mutant** strains, but not from the parent strain possessing proteases.

L9 ANSWER 7 OF 40 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:315929 CAPLUS
DOCUMENT NUMBER: 129:77202
TITLE: Vectors for expressing T7 epitope- and His6 affinity-tagged **fusion** proteins in S. cerevisiae
AUTHOR(S): Enomoto, Shinichiro; Chen, Guanghui; Berman, Judith
CORPORATE SOURCE: University of Minnesota, St. Paul, MN, USA
SOURCE: BioTechniques (1998), 24(5), 782-784, 786, 788
CODEN: BTNQDO; ISSN: 0736-6205
PUBLISHER: Eaton Publishing Co.

PUBLISHER: Eaton Publishing Co.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB We have constructed a series of vectors (YGALSETs) for the expression of epitope- and affinity-tagged **fusion** proteins in yeast cells using the regulated GAL10 promoter. **Fusion** proteins produced from YGALSET plasmids include a leader peptide at the N terminus that encodes both a T7 gene 10 epitope tag and a His6 affinity tag. The YGALSET vector series includes centromere plasmids for low-copy plasmid maintenance and 2 .mu. episomal plasmids for multicopy plasmid maintenance and four different selectable markers: TRP1, URA3, LEU2 and HIS3. We also provide a convenient approach for transferring cloned genes from a bacterial expression vector into YGALSET vectors by in vivo recombination and a rapid method to screen directly for clones that express the **fusion** protein of interest.

L9 ANSWER 8 OF 40 MEDLINE
ACCESSION NUMBER: 97472174 MEDLINE
DOCUMENT NUMBER: 97472174
TITLE: Mechanism of suppression of piperacillin resistance in enterobacteria by tazobactam.
AUTHOR: Kadima T A; Weiner J H
CORPORATE SOURCE: Department of Biochemistry, University of Alberta, Edmonton, Canada.
SOURCE: ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, (1997 Oct) 41 (10) 2177-83.
Journal code: 6HK. ISSN: 0066-4804.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801
ENTRY WEEK: 19980104
AB Resistance to piperacillin in several isolates of Citrobacter freundii and Enterobacter cloacae was investigated and confirmed to occur at a frequency of 10(-7) to 10(-6). Development of resistance to piperacillin was significantly suppressed by tazobactam but not by clavulanic acid. To elucidate the mechanism by which resistance suppression occurs, the effect of piperacillin plus tazobactam on the induction of AmpC **beta-lactamase** was analyzed by monitoring the beta-galactosidase activity of an inducible ampC-lacZ gene **fusion** in Escherichia coli. The combination exerted no inhibitory effect on AmpC **beta-lactamase** induction. Tazobactam also had no effect on the accumulation of a key intermediate in the AmpC **beta-lactamase** induction pathway, 1,6-anhydromurotripeptide, in an ampD **mutant** strain of E. coli. However, the addition of tazobactam to liquid cultures of E. cloacae 40001 in the presence of piperacillin at four times the MIC caused a delay in the recovery of the culture to piperacillin-induced stress. At 16 times the MIC, a complete suppression of regrowth occurred. Analysis of culture viability on piperacillin plates showed that the culture recovery was due to growth by moderately resistant mutants preexisting in the cell population, which at 16 times the MIC became susceptible to the combination. Evidence from the kinetics of inhibition of the E. cloacae 40001 AmpC **beta-lactamase** by clavulanic acid, sulbactam, and tazobactam and from the effects of these drugs on the frequency of resistance to piperacillin suggests that the suppressive effect of tazobactam on the appearance of resistance is primarily mediated by the **beta-lactamase** inhibitory activity.

L9 ANSWER 9 OF 40 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1997:755270 CAPLUS
TITLE: The 5' untranslated region of the Escherichia coli outer membrane protein gene, ompA: evidence for structural and functional regulators of mRNA degradation
AUTHOR(S): Arnold, Todd E.; Yu, Jeanne J.; Belasco, Joel G.
CORPORATE SOURCE: Department of Microbiology and Molecular Genetics, Boston, MA, 02115, USA
SOURCE: Nucleic Acids Symp. Ser. (1997), 36(Symposium on RNA Biology, II. RNA: Tool and Target, 1997), 119
CODEN: NACSD8; ISSN: 0261-3166
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The highly-structured 5' untranslated region of the gene encoding the major outer membrane protein A (ompA) of Escherichia coli encodes elements which regulate transcript longevity and protein synthesis. The 5' UTR of the ompA mRNA functions to stabilize its own transcript as well as to prolong the lifespan of a no. of heterologous E. coli transcripts to which it is fused, for example .**beta**.-**lactamase** (bla). To elucidate the role(s) played by the elements in the ompA 5'-UTR, mutational anal. has been performed on structural and functional domains of this region. Removal of the 5'-terminal stem-loop from the 5' UTR of ompA generates a labile **variant** mRNA whose in vivo half-life is reduced 3-fold. Attachment of variously-sized 5'-terminal stem-loops, which form in vivo, differ in their capacity to stabilize a labile ompA mRNA **mutant**; and, short self-complementary sequences that lack the thermodn. energy to form in vivo, are unable to stabilize labile ompA transcripts to which they are fused. In an ompA/bla **fusion** mRNA, mutations that significantly decrease or prematurely terminate OmpA/Bla **fusion** protein translation also markedly reduce this hybrid transcript's half-life relative to its efficiently translated counterpart. Deletion of the major endo- and exoribonucleases of E. coli, has demonstrated that each of these labile **variant** ompA mRNAs is decayed via different pathways and evidence is presented indicating that a major effector of ompA mRNA degrdn. is a RNase(s) yet to be characterized.

L9 ANSWER 10 OF 40 MEDLINE
ACCESSION NUMBER: 96310373 MEDLINE
DOCUMENT NUMBER: 96310373
TITLE: Optimized BlaM-transposon shuttle mutagenesis of Helicobacter pylori allows the identification of novel genetic loci involved in bacterial virulence.
AUTHOR: Odenbreit S; Till M; Haas R
CORPORATE SOURCE: Max-Planck-Institut fur Biologie, Abteilung Infektionsbiologie, Tubingen, Germany.
SOURCE: MOLECULAR MICROBIOLOGY, (1996 Apr) 20 (2) 361-73.
Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Z68311; GENBANK-Z68312; GENBANK-Z68313
ENTRY MONTH: 199702
AB Helicobacter pylori is an important etiologic agent of gastroduodenal disease in humans. In this report, we describe a general genetic approach for the identification of genes encoding exported proteins in H. pylori. The novel TnMax9 mini-blaM transposon was used for insertion mutagenesis

The novel TnMax9 mini-blaM transposon was used for insertion mutagenesis of a H. pylori gene library established in Escherichia coli. A total of 192 E. coli clones expressing active **beta-lactamase fusion** proteins (BlaM+) were obtained, indicating that the corresponding target plasmids carry H. pylori genes encoding putative extracytoplasmic proteins. Natural transformation of H. pylori P1 or P12 using the 192 **mutant** plasmids resulted in 135 distinct H. pylori **mutant** strains (70%). Screening of the H. pylori collection of **mutant** strains allowed the identification of **mutant** strains impaired in motility, in natural transformation competence and in adherence to gastric epithelial cell lines. Motility mutants could be grouped into distinct classes: (i) **mutant** strains lacking the major flagellin subunit FlaA and intact flagella (class I); (ii) **mutant** strains with apparently normal flagella, but reduced motility (class II), and (iii) **mutant** strains with obviously normal flagella, but completely abolished motility (class III). Two independent mutations that exhibited defects in natural competence for genetic transformation mapped to different genetic loci. In addition, two independent **mutant** strains were isolated by their failure to bind to the human gastric carcinoma cell line KatoIII. Both **mutant** strains carried a transposon in the same gene, 0.8 kb apart, and showed decreased autoagglutination when compared to the wild-type strain.

L9 ANSWER 11 OF 40 MEDLINE

ACCESSION NUMBER: 95394841 MEDLINE
DOCUMENT NUMBER: 95394841
TITLE: Extracellular secretion of pullulanase is unaffected by minor sequence changes but is usually prevented by adding reporter proteins to its N- or C-terminal end.
AUTHOR: Sauvonnet N; Poquet I; Pugsley A P
CORPORATE SOURCE: Unite de Genetique Moleculaire (Centre National de la Recherche Scientifique URA1149, Institut Pasteur, Paris, France.
SOURCE: JOURNAL OF BACTERIOLOGY, (1995 Sep) 177 (18) 5238-46. Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199512

AB Linker insertions in the pullulanase structural gene (pulA) were examined for their effects on pullulanase activity and cell surface localization in Escherichia coli carrying the cognate secretion genes from Klebsiella oxytoca. Of the 23 insertions, 11 abolished pullulanase activity but none were found to prevent secretion. To see whether more drastic changes affected secretion, we fused up to five reporter proteins (E. coli periplasmic alkaline phosphatase, E. coli periplasmic maltose-binding protein, periplasmic TEM **beta-lactamase**, Erwinia chrysanthemi extracellular endoglucanase Z, and Bacillus subtilis extracellular levansucrase) to three different positions in the pullulanase polypeptide: close to the N terminus of the mature protein, at the C terminus of the protein, or at the C terminus of a truncated pullulanase **variant** lacking the last 256 amino acids. Only 3 of the 13 different hybrids were efficiently secreted: 2 in which **beta-lactamase** was fused to the C terminus of full-length or truncated pullulanase and 1 in which maltose-binding protein was fused close to the N terminus of pullulanase. Affinity-purified endoglucanase-pullulanase and pullulanase-endoglucanase hybrids exhibited apparently normal levels of pullulanase activity, indicating that the conformation of the pullulanase segment of the hybrid

indicating that the conformation of the pullulanase segment of the hybrid had not been dramatically altered by the presence of the reporter. However, pullulanase-endoglucanase hybrids were secreted efficiently if the endoglucanase component comprised only the 60-amino-acid, C-terminal cellulose-binding domain, suggesting that at least one factor limiting hybrid protein secretion might be the size of the reporter.

L9 ANSWER 12 OF 40 MEDLINE
ACCESSION NUMBER: 95103534 MEDLINE
DOCUMENT NUMBER: 95103534
TITLE: Development of a humanized disulfide-stabilized anti-p185HER2 Fv-**beta-lactamase** **fusion** protein for activation of a cephalosporin doxorubicin prodrug.
AUTHOR: Rodrigues M L; Presta L G; Kotts C E; Wirth C; Mordenti J; Osaka G; Wong W L; Nuijens A; Blackburn B; Carter P
CORPORATE SOURCE: Department of Cell Genetics, Genetech Inc, South San Francisco, CA 94080-4990.
SOURCE: CANCER RESEARCH, (1995 Jan 1) 55 (1) 63-70. Journal code: CNF. ISSN: 0008-5472.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199504
AB The humanized anti-p185HER2 antibody, humAb4D5-8, has completed Phase II clinical trials for p185HER2-overexpressing breast cancer. Here, this antibody is used as a building block to engineer a disulfide-linked Fv (dsFv) **beta-lactamase** **fusion** protein for use in antibody-dependent enzyme-mediated prodrug therapy using cephalosporin-based prodrugs. Three Fv variants were designed with an interchain disulfide bond buried at the VL/VH interface and secreted from Escherichia coli. One **variant**, dsFv3 (VL L46C VH D101C0, has similar affinity for antigen (Kd = 0.7 nM) as the wild-type Fv and was used to construct a **fusion** protein in which **beta-lactamase**, RTEM-1, is joined to the carboxy terminus of VH. The dsFv3-**beta-lactamase** **fusion** protein secreted from E. coli efficiently activates a cephalothin doxorubicin prodrug (PRODOX, kcat/km = 1.5 x 10(5) s-1 M-1). PRODOX is approximately 20-fold less toxic than free doxorubicin against breast tumor cell lines SK-BR-3 and MCF7, which express p185HER2 at elevated and normal levels, respectively. Prebinding the dsFv3-**beta-lactamase** **fusion** protein specifically enhances the toxicity level of PRODOX to that of doxorubicin against SK-BR-3 but not MCF7 cells. The **fusion** protein retains both antigen-binding plus kinetic activity in murine serum and is cleared rapidly as judged by pharmacokinetic analysis in nude mice (initial and terminal half-lives of 0.23 and 1.27 h, respectively). Development and characterization of the dsFv3-**beta-lactamase** **fusion** protein is an important step toward targeted prodrug therapy of p185HER2-overexpressing tumors.

L9 ANSWER 13 OF 40 MEDLINE
ACCESSION NUMBER: 95276466 MEDLINE
DOCUMENT NUMBER: 95276466
TITLE: Expression of truncated and full-length forms of the Lyme disease Borrelia outer surface protein A in Escherichia coli.
AUTHOR: Hansson L; Noppa L; Nilsson A K; Stromqvist M; Bergstrom S
CORPORATE SOURCE: Symbicom AB, Umea, Sweden..
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (1995 Feb) 6 (1)

SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (1995 Feb) 6 (1) 15-24.
Journal code: BJV. ISSN: 1046-5928.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199509

AB The lipidated major outer surface protein, OspA, of the Lyme disease spirochaete may be important in the pathogenesis during Lyme borreliosis. To produce sufficient amounts of purified OspA variants to perform pathogenesis studies in vivo and in vitro, different recombinant OspA expression systems in Escherichia coli were constructed. Recombinant OspA variants were produced as a full-length molecule, as a truncated **variant** lacking the N-terminal lipidated cysteine, or as a **fusion** protein with the synthetic dimer of Staphylococcus aureus protein A IgG binding domain (ZZ). In order to produce the full-length protein, four different promoters were evaluated. These were combined with either the OspA original signal sequence or the E. coli Brauns lipoprotein signal sequence, lpp. The most efficient production of the full-length lipidated OspA was mediated by the constitutive **beta-lactamase** promoter in combination with lipoprotein signal sequences. For production of truncated nonlipidated OspA the S. aureus protein A signal sequence was ligated to the OspA open reading frame. Alternatively, truncated OspA was produced intracellularly using expression vectors that lack signal sequences. Production of nonlipidated protein with a heterologous signal peptide resulted in a soluble protein located mainly in the periplasm and in the culture medium. The full-length lipidated OspA, on the other hand, was associated mainly with the membrane fraction. The production level of the lipidated recombinant OspA was much lower than the level obtained with the truncated ZZ-OspA **fusion** protein.

L9 ANSWER 14 OF 40 MEDLINE
ACCESSION NUMBER: 94344037 MEDLINE
DOCUMENT NUMBER: 94344037
TITLE: Transcription and expression analysis, using lacZ and phoA gene fusions, of Mycobacterium fortuitum **beta-lactamase** genes cloned from a natural isolate and a high-level **beta-lactamase** producer.
AUTHOR: Timm J; Perilli M G; Duez C; Trias J; Orefici G; Fattorini L; Amicosante G; Oratore A; Joris B; Fr`ere J M; et al
CORPORATE SOURCE: Unite de Genetique Mycobacterienne, CNRS URA 1300, Institut Pasteur, Paris, France..
SOURCE: MOLECULAR MICROBIOLOGY, (1994 May) 12 (3) 491-504.
Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L25634
ENTRY MONTH: 199411

AB The gene encoding a class A **beta-lactamase** was cloned from a natural isolate of Mycobacterium fortuitum (blaF) and from a high-level amoxicillin-resistant **mutant** that produces large amounts of **beta-lactamase** (blaF*). The nucleotide sequences of the two genes differ at 11 positions, including two in the region upstream from the coding sequence. Gene fusions to Escherichia coli lacZ and transcription and expression analysis of the cloned genes in Mycobacterium smegmatis indicated that high-level production of the

Mycobacterium smegmatis indicated that high-level production of the **beta-lactamase** in the **mutant** is mainly or wholly due to a single base pair difference in the promoter. These analyses also showed that transcription and translation start at the same position. A comparison of the amino acid sequence of BlaF, as predicted from the nucleotide sequence, with the determined N-terminal amino acid sequence indicated the presence of a typical signal peptide. The **fusion** of blaF (or blaF*) to the E. coli gene phoA resulted in the production of BlaF-PhoA hybrid proteins that had alkaline phosphatase activity. These results demonstrate that phoA can be used as a reporter gene for studying protein export in mycobacteria.

L9 ANSWER 15 OF 40 MEDLINE
ACCESSION NUMBER: 94344033 MEDLINE
DOCUMENT NUMBER: 94344033
TITLE: **beta-Lactamase** topology probe analysis of the OutO NMePhe peptidase, and six other Out protein components of the Erwinia carotovora general secretion pathway apparatus.
AUTHOR: Reeves P J; Douglas P; Salmond G P
CORPORATE SOURCE: Department of Biological Sciences, University of Warwick, Coventry, UK.
SOURCE: MOLECULAR MICROBIOLOGY, (1994 May) 12 (3) 445-57. Journal code: MOM. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
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AB The out gene cluster of Erwinia spp. encodes the proteins of the general secretory pathway (GSP) apparatus that is required for pectinase and cellulase secretion. We have used fusions between Erwinia carotovora subsp. carotovora (Ecc) out genes and the topology probe blaM to assess the ability of Out protein regions to export BlaM across the cytoplasmic membrane in Escherichia coli and Ecc. For the outO gene product (an NMePhe peptidase), seven transmembrane regions have been identified and one more is predicted. The region of OutO with the highest level of hydrophilicity is likely to exist as a large cytoplasmic loop, located between two hydrophobic domains, and is positioned towards the N-terminus of the protein. When BlaM was fused on the C-terminal side of the last hydrophobic stretch of OutO, the resulting hybrid protein transferred the BlaM moiety to the periplasm whilst retaining OutO activity. Removal of a portion of this hydrophobic stretch resulted in the loss of OutO activity, suggesting that there are tight constraints on the topological integrity of OutO for maintaining catalytic function. When outG, -H, -I, -J, -K and -N were fused to blaM, the resulting phenotype suggested that the majority of each protein was targeted to the periplasm. Our results indicate that these six Out proteins, when produced by E. coli or Ecc, each adopt, at least temporarily, a type II bitopic conformation in the cytoplasmic membrane. For OutG, -H, -I and -J this probably represents the membrane topology prior to processing by OutO in Ecc. When produced in vivo from a T7 gene 10 promoter construct, the outG product was processed in Ecc whereas the outO **mutant** RJP249 failed to process pre-OutG. BlaM fusions positioned on the C-terminal side of the hydrophobic stretches of pre-OutG, -H, -I, and -J were processed by wild-type Ecc but not RJP249 or E. coli DH1. Thus the periplasmic domains of these proteins play no role in the peptidase cleavage reaction. An OutG-BlaM **fusion** construct was used to demonstrate NMePhe peptidase activity in other bacterial strains including E. carotovora subsp. carotovora (ATCC39048), E. carotovora subsp. atroseptica (SCRI1043) and Erwinia chrysanthemi

E. carotovora subsp. atroseptica (SCRI1043) and Erwinia chrysanthemi (3937).

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